THE CHARACTERISTIC FEATURES OF FUNGI TAKING PART IN THE DECOMPOSITION OF BEECH LITTER*

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The decomposition of beech litter in relation to its microbial population has been outlined in previous papers (Saitô 1956, 1957). The present work deals with the specific features and behaviours of fungi playing a prominent rôle in the decomposition of the litter.

MATERIAL AND METHODS

The litter investigated was that of the same beechwood in the neighbourhood of Tsuta hot spring, Aomori Prefecture, as described in the previous papers.

Of some twenty isolates from the beech litter of filamentous fungi, *Absidia glauca*, *Mucor ramannianus*, *Mucor* sp., *Trichoderma viride*, *Penicillium lapidosum* and *Penicillium herquei* were chosen for study, since these have been found to belong to a group of fungi of prime importance in decomposing the litter.

According to field observations in early August, last year, the production of basidiomycetous fruit bodies was intense in *Collybia confluens*(?), moderate in *Collybia* sp., and sparse both in *Mycena* sp. and in an unidentified agaric designated for the present as "Basidiomycete D". Attempts were made to isolate these basidiomycetes in the following way, but the spore of *Collybia confluens*, which is widespread in the litter, failed to germinate on varied culture media, while the three others were much less exacting in food requirement and readily grown on a variety of media. Spore was collected on a sheet of sterilized paper from a pileus placed upon it. It gives rise to a mycelium on a plate of glucose-peptone-yeast-agar. Beside those isolated from the litter itself, *Merulius lacrymans*, *Polyporus betulinus*, *Collybia velutipes* and *Fomes fomentarius* were also used in some experiments.

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THE CAPACITY OF DECOMPOSING LEAVES OF DIFFERENT FUNGI

As an index of the capacity of decomposing leaves of a fungus, the loss in weight of the original *brown leaf*, when inoculated with it, was estimated. Thus, a square piece with sides of 1 cm. of the brown leaf was inoculated with a definite amount of mycelia at four spots on its surface and incubated at 26°C. in a moist chamber for 17 days. As will be seen from Table 1, basidiomycetes

Table 1

The loss in weight (%) of the brown leaf when inoculated with different fungi

Collybia sp.	10.6
Mycena sp.	13.4
Basidiomycete D	17.9
Absidia glauca	6.2
Mucor ramannianus	5.8
Mucor sp.	4.0
Trichoderma viride	10.0
Penicillium lapidosum	5.7

cause by far larger losses, compared with filamentous fungi with the exception of *Trichoderma viride*.

THE FUNGAL GROWTH ON STERILE AND CONTAMINATED LEAVES

It has been mentioned in an earlier paper, that, in the course of the decomposition of the litter, the original *brown leaves* having fallen on the ground first turn yellowish (*yellowish leaves*) and then become heavily infected with basidiomycetous mycelia (*mouldy leaves*), while those having got rid of the basidiomycetous attack remain as *greyish-brown* leaves.

The behaviours of fungi on both sterilized and contaminated leaves at the foregoing stages of decomposition, which were inoculated with them, were compared. Sterilization was performed by autoclaving at 120°C. for 20 minutes and by exposing to propylene oxide vapour for 48 hours. The contaminated leaves were partly those taken from the litter directly and with their own microflora and partly those which were soaked into a litter suspension after dried in air. The brown, yellowish, mouldy and greyish-brown leaves treated in such ways were inoculated with *Collybia* sp., Basidiomycete D and *Trichoderma viride* and after over five days of incubation the mycelial development was estimated visually (Table 2).

As a rule all of the three fungi show a better growth on the sterilized leaves, particularly on the autoclaved, than on the contaminated ones. Exposure

to propylene oxide vapour was found not to result in a complete sterilization. And, it is very likely that autoclaving causes a more or less change in the physical and chemical conditions in the leaves, which may lead to an increase in the available organic matter.

Table 2

The fungal growth on sterile and contaminated leaves as indicated with numerical scales, the larger the better

		Brown leaf	Yellowish leaf	Mouldy leaf	Greyish-brown leaf
Collybia sp.	Autoclaved	7	7	10	7
	Fumigated	6	6	8	4
	Deliberately contaminated	4	2	2	4
	Natural	4	2	0	0
Basidiomycete D	Autoclaved	8	8	10	8
	Fumigated	4	4	6	5
	Deliberately contaminated	6	2	2	5
	Natural	6	2	0	1
Trichoderma viride	Autoclaved	6	8	10	6
	Fumigated	4	7	8	5
	Deliberately contaminated	4	6	8	4
	Natural	4	2	0	2

It will be seen that, in the yellowish and mouldy leaves, the previous contamination is admittedly unfavourable for the growth of basidiomycetes. Since the good growth of basidiomycetes on these leaves sterilized in advance implies that the substrate itself is not unsuitable, the basidiomycetous mycelia no doubt fail to develop in the presence of competitors.

The brown leaves with little contamination show a fairly good development of fungi, with which they are inoculated, even in the untreated ones, because probably of the presence of few competitors. In contrast to them, the previously most heavily infected natural mouldy leaves preclude most strongly the growth of the fungi. It is the case even with those which have been overgrown with *Collybia* sp. under the natural conditions and are inoculated with the same fungus anew. As has been reported previously, the greyish-brown leaves are comparatively rich in filamentous fungi, which will compete with the inocula.

Park (1955) has described that patially decomposed plant materials can be utilized in the soil by both native and alien fungi, but only the former are able to make the fullest use of them. Garrett (1950) put forward the concept of the competitive saprophytic ability, which was supported by Park. According to Garrett, the root-infecting fungi having the competitive saprophytic ability to a less degree are unable to compete in establishing themselves as soil saprophytes with the soil-inhabiting fungi, which are capable of decomposing plant debris rapidly and completely, grow vigorously, and excrete antibiotic toxins and

moreover are tolerant of them. In reality a high competitive saprophytic ability is to be assigned to the basidiomycetes in beech litter.

THE FUNGAL GROWTH ON THE BROWN LEAVES FROM WHICH DIFFERENT SOLUBLE FRACTIONS HAVE BEEN REMOVED

To analyse the effect of each of different soluble fractions of the brown leaves upon the fungal growth, they were cultivated on those from which various fractions were removed. Thus, powdered brown leaves passed through a 1 mm.-mesh sieve were treated with (1) cold water, (2) hot water, (3) alcohol-benzene, (4) hot water and alcohol-benzene, or (5) hot-water, alcohol-benzene and 2 % hydrochloric acid. A definite amount of the treated leaves was moistened with water twice as much as its weight and placed on a slide glass in a humid atmosphere in a petri dish. After autoclaved at 120°C. for 20 minutes, it was inoculated with a piece of infected agar from an agar plate and incubated at 26°C. Table 3 shows the development of the fungi after 5 days.

Table 3

The fungal growth on the brown leaves destitute of different fractions

	Original	Treated with cold water	Treated with hot water	Treated with alcohol-benzene	Treated with hot water & alcohol-benzene	Treated with hot water, alcohol-benzene & 2% HCl
Penicillium lapidosum	10	3	less than 1	3	less than 0.5	±
Penicillium herquei	10	3	less than 1	3	less than 0.5	±
Absidia glauca	10	2	less than 1	3	less than 0.5	±
Mucor ramannianus	10	3	less than 1	1	less than 0.5	±
Collybia sp.	10	8	7	10	6	2
Basidiomycete D	10	6	6	10	6	2
Merulius lacrymans	10	8	6	8	6	2
Polyporus betulinus	10	8	6	7	6	1
Collybia velutipes	7	6	4	10	5	2
Fomes fomentarius	7	8	4	10	6	2

Those treated with cold and hot water, particularly those with the latter, i. e. those destitute of the water-soluble fraction, show a striking inhibition of the growth of filamentous fungi, but permit a relatively good development of basidiomycetes. The removal of the alcohol-benzene-soluble fraction is also detrimental to the filamentous fungi, whereas it exerts little or no effect upon the basidiomycetes and is sometimes even promotive, for their growth. As a consequence the removal of both water- and alcohol-benzene-soluble fractions

suppresses the growth of the former a great deal, and further treatment with hydrochloric acid arrests their growth almost completely. It is of special interest that the basidiomycetes are able to grow to a little extent even after the acid treatment.

According to Waksman (1932), sugar, starch and simple carbohydrates in general are mostly soluble in cold water, though better in hot water. The alcohol-benzene fraction comprises oil, fat, wax, resin etc. Melin (1930), Tsutsumi (1956) and others have reported that the ether-soluble fraction of coniferous needles is unlikely to retard their decomposition. From the present findings alcohol-benzene-soluble substances seem to be important for the growth of filamentous fungi. Those treated with both water and alcohol-benzene are thought to contain mainly hemicellulose, cellulose, lignin etc., and digestion with hydrochloric acid will remove hemicellulose.

DIRECT MICROSCOPIC OBSERVATION OF THE INFECTED LEAVES

The mouldy leaves, which are overgrown with basidiomycetous mycelia and under the most active decomposition, were examined directly under a microscope. Based on the degree of decomposition, three stages may further be distinguished. The least decomposed first stage is characterized by the most active state of the basidiomycetes. As the decomposition proceeds their activity sinks gradually (the second stage), and finally it becomes feeble (the third stage). In Table 4,

Table 4

The number of bacteria per g. of dry mouldy leaves

The first stage	5.0×10^8
The second stage	3.8×10^9
The third stage	7.5×10^9

the bacterial number determined by the Conn's direct microscopic method is given of the mouldy leaves at the three stages, which are being attacked by *Collybia* sp. under the natural conditions. At all of the stages a large amount of basidiomycetous mycelia associated with a vigorous growth of bacteria is noticed. Even at the first stage the bacterial count per g. of dry leaf amounts to about five hundred millions, some of them aggregating around the mycelia. The viable basidiomycetous mycelia filled with protoplasm, which are characteristic of the first stage, undergo lysis at the second stage and empty spaces in hyphae increase with the lapse of time. Especially in the third stage the lytic process is pronounced and leads to a complete degradation of the cell contents,

only the cell wall remaining. Meanwhile there is a marked rise in the number of bacteria up to seventy-five hundred millions with the loss of the mycelial activity. It follows from this, that the basidiomycetous mycelia are likely to be very susceptible to the bacterial attack. Apart from the nutritional value of the degradation products of leaves, the mycelial contents probably serve as the bacterial food. In this connection, Jensen (1934), Ziemiecka (1935), Starkey (1938) and others have observed the bacterial attack on fungal mycelia developed on buried slide glasses.

The majority of bacteria found in great abundance in the mouldy leaves are short or coccoid rods, elongated rods being also noticed. Some ten strains of the dominant bacteria were isolated by the use of albumen-agar, glucose-peptone-yeast-agar and broth-agar. Most of them are gram-negative rods of varying length. They were employed as the test bacteria in the experiment regarding antibiotics to be referred to below.

THE ANTIBIOTIC CAPACITY OF THE ISOLATED FUNGI

The fungi isolated from the litter were tested for their antibiotic capacity by screening (Table 5). The strains of *Collybia* sp. and *Mycena* sp. were found

Table 5

Antibacterial spectra of three species of basidiomycetes as indicated with the widths (mm.) of inhibition zones

	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*
Collybia sp.	0	10	15
Mycena sp.	0	6	3
Basidiomycete D	0	4	0

to exert a bacteriostatic action against the gram-positive *Bacillus subtilis* and *Staphylococcus aureus*. The latter bacterium is also susceptible to Basidiomycete D. In addition, ten bacterial isolates from the mouldy leaves were tested, but none of them was sensitive to these fungi. The filamentous fungi showed no appreciable inhibitory effects upon the foregoing bacteria.

Wright (1956a, b) has brought out that the production of antibiotic substances is far higher in straw and viable seeds than in the surrounding soil. Whether or not the antibacterial substances produced by the basidiomycetes mentioned above are produced in mouldy leaves under the natural conditions, is to be studied later on.

SUMMARY

1. Brown leaves of beech having just fallen and with little infection show experimentally a larger loss in weight on basidiomycetous infection than on infection with filamentous fungi in general.

2. It is difficult to inoculate with fungi the leaves under decomposition, where a luxuriant microbial population has already established itself. If the natural microflora is removed by sterilization, fungal inocula develop freely.

3. Removal of the water- or the alcohol-benzene-soluble fraction affects the growth of filamentous fungi remarkably. However, basidiomycetes are affected by the absence of the water-soluble fraction to a far less extent and are practically indifferent to the alcohol-benzene-soluble fraction. An additional treatment with HCl of the leaves suppresses the growth of the former nearly completely, whereas the latter can develop upon such leaves though slightly.

4. Basidiomycetous mycelia attacking leaves are finally broken down by bacteria associated with them.

5. Basidiomycetes isolated from the litter show an antibacterial capacity.

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